to collect mosquito saliva, directly concentrate proteins by trichloroacetic acid precipitation, and fractionate them by non-denaturing PAGE. We performed immunoblot analysis with these proteins and sera from 200 Thai children who had been diagnosed with DF, DHF, or no dengue virus infection. We showed a possible correlation between the presence of antibodies to certain Aedes aegypti saliva proteins and severity of disease. These results suggest that the immune response to vector mosquito salivary proteins might play a role in the outcome of this arboviral disease.

23rd Annual Meeting of the American Society of Virology. Montreal, Canada. 10-14 July 2004. Abstract no. W23-6:121.

PROBLEMS ENCOUNTERED IN THE MOLECULAR DETECTION OF DENGUE VIRUSES

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The dengue virus (DENV) molecular typing method, reverse transcriptase - polymerase chain reaction (RT-PCR), described by Lanciotti (Lanciotti et al., 1992) has been used in many laboratories for detecting DENV infection. However, we currently have observed some non-specific results when using this method to detect DENV infection. Some samples showed evidence for multiple infections by two different dengue serotypes, in most cases, DENV-1 plus another serotype. Additionally, some samples gave positive results for DENV-1 by serological tests or show a right size of DNA fragment amplified from the first-round PCR, but negative results by the nested PCR (the second-round) in agarose gel. These non-specific results thus interfered with our ability to accurately detect DENV infection by using this technique. We designed a few of new primers based on our Thai sequence database of DENV variants to modify Lanciotti's RT-PCR and eliminate non-specific reactions in detecting Thai DENV variants.

Dengue Virus Research Workshop. Geneva, Switzerland. 5 October 2004.

A RANDOMIZED, PLACEBO-CONTROLLED, STUDY OF NON-PEGYLATED AND PEGYLATED FORMS OF RECOMBINANT HUMAN INTERFERON-α-2A FOR SUPPRESSION OF DENGUE VIREMIA IN RHESUS MACAQUES

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Infection with any dengue viruses can produce a spectrum of disease, ranging from a mild febrile illness, to classic dengue fever, to the most severe form, dengue hemorrhagic fever (DHF). There is growing evidence that viremia levels and the overall viral burden are greatest in DHF. A therapeutic intervention to suppress viremia early in dengue infection could potentially ameliorate severe disease. Two sequential studies examined the effects of recombinant interferon-α (rIFN-α)-2a (Roferon®-A) and pegylated rIFN-α-2a (PEGASYS®) on dengue-2 (D2) viremia

in Rhesus macaques. Flavivirus-naïve macaques were inoculated with D2 virus and randomized in the first study to receive a single dose of Roferon®-A (10 MU/m^2) or placebo, and in the second study to receive PEGASYS® (6 μg/kg) or placebo, one day after the onset of viremia. Serial daily viremia levels were measured, and convalescent D2 virus neutralizing antibody titers were determined. Compared to placebo, Roferon®-A temporarily suppressed D2 virus replication and delayed the time to peak viremia by a median of 2 days. Mean peak serum viremia levels and area under the curve (AUC) virus concentrations were not different between the two groups. This finding led to a study of pegylated rIFN-α. PEGASYS® produced a 1-log drop in mean daily viremia levels over 4 days when compared to placebo. Peak viremia levels were not significantly affected, but D2 virus AUC and elimination $t_{1/2}$ trended lower in the PEGASYS® group. There were no significant differences in D2 virus $PRNT_{50}$ between two groups at 30 and 90 days post-infection. Rapid identification of dengue viremic patients early in illness may provide an opportunity to suppress viremia and ameliorate subsequent disease severity. A single injection of PEGASYS®, or a combination of PEGASYS® and Roferon®-A, early in dengue illness should be further investigated.

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RELATIONSHIP OF PREEXISTING DENGUE VIRUS (DV) NEUTRALIZING ANTIBODY LEVELS TO VIREMIA AND SEVERITY OF DISEASE IN A PROSPECTIVE COHORT STUDY OF DV INFECTION IN THAILAND

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Background: Infection with any 1 of the 4 dengue viruses (DVs) can produce several illnesses, ranging from a mild febrile illness to classic dengue fever (DF) to dengue hemorrhagic fever (DHF), a potentially life-threatening disease. Most DHF cases occur after sequential heterotypic DV infections. The role of preexisting humoral immunity in modifying severity of dengue disease is not well understood.

Methods: We conducted a prospective cohort study of children in a region where dengue disease is hyperendemic and examined the role of preexisting neutralizing anti-DV anti-bodies (Abs) in modifying secondary dengue-3 virus (D3V), dengue-2 virus (D2V), and dengue-1 virus (D1V) infections.

Results: In secondary D3V infection, higher levels of preexisting neutralizing Ab directed against D3V (reference virus strain and patient's virus isolate) were associated with lower viremia levels and milder disease. Preexisting neutralizing Ab levels against D2V were not associated with severity of secondary D2V infection. The levels of preexisting neutralizing Ab against the infecting virus isolates were not associated with viremia levels in secondary D2V or D1V infections.